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L2: Entry 1 of 1 File: PGPB Dec 19, 2002

DOCUMENT-IDENTIFIER: US 20020192707 A1
TITLE: Methods for creating a compound library

Pre-Grant Publication (PGPub) Document Number (1):
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Detail Description Paragraph (16):
[0051] Sample requirements can be reduced even further if WaterLOGSY methods are used as an alternative to the relaxation-editing method described above to detect the binding interaction. WaterLOGSY is described in more detail in C. Dalvit et al., J. Biomol. NMR, 18, 65-68 (2000).

Detail Description Paragraph (58):
[0084] Changes in chemical shifts, relaxation properties or diffusion coefficients that occur upon the interaction between a protein and a small molecule have been documented for many years (for recent reviews see M. J. Shapiro et al., Curr. Opin. Drug. Disc. Dev., 2, 396 (1999); J. M. Moore, Biopolymers, 51, 221 (1999); and B. J. Stockman, Prog. NMR Spectr., 33, 109 (1998)). Observables typically used to detect or monitor the interactions are chemical shift changes for the ligand or isotopically-enriched protein resonances (J. Wang et al., Biochemistry, 31, 921 (1992)), or line broadening (D. L. Rabenstein, et al., J. Magn. Reson., 34, 669 (1979); and T. Scherf et al., Biophys. J., 64, 754 (1993)), change in sign of the NOE from positive to negative (P. Balaram et al., J. Am. Chem. Soc., 94, 4017 (1972); and A. A. Bothner-By et al., Ann. NY Acad. Sci. 222, 668 (1972)), or restricted diffusion (A. J. Lennon et al., Biophys., J. 67, 2096 (1994)) for the ligand. For the most part, these studies have focussed on protein/ligand systems where the small molecule was already known to be a ligand or was assumed to be one. In the last several years, however, the work of the Fesik (S. B. Shuker et al., Science, 274, 1531 (1996); and P. J. Hajduk et al., J. Am. Chem. Soc., 119, 12257 (1997)), Meyer (B. Meyer et al., Eur. J. Biochem., 246, 705 (1997)), Moore (J. Fejzo et al., Chem. Biol., 6, 755 (1999)), Shapiro (M. Lin et al., J. Org. Chem., 62, 8930 (1997)), and Dalvit (C. Dalvit et al., J. Biomol NMR, 18, 65-68 (2000)) labs has demonstrated the applicability of these same general methods as a screening tool to identify ligands from mixtures of small molecules.

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L3: Entry 1 of 1 File: PGPB Dec 19, 2002

DOCUMENT-IDENTIFIER: US 20020192707 A1
TITLE: Methods for creating a compound library

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Summary of Invention Paragraph (12):
[0011] Sample requirements can be reduced even further if WaterLOGSY (water-ligand observation with gradient spectroscopy) methods are used as an alternative to the relaxation-editing method described above to detect the binding interaction.

Summary of Invention Paragraph (13):
[0012] The present invention provides yet another method of identifying a compound that binds to a target molecule (e.g., protein). This method includes: providing a plurality of mixtures of test compounds, each mixture being in a sample reservoir; introducing a target molecule into each of the sample reservoirs to provide a plurality of test samples; providing a nuclear magnetic resonance spectrometer equipped with a flow-injection probe; transferring each test sample from the sample reservoir into the flow-injection probe; collecting a WaterLOGSY nuclear magnetic resonance spectrum (preferably, a 1D WaterLOGSY nuclear magnetic resonance spectrum) on each sample in each reservoir; and analyzing the spectra of each sample to distinguish binding compounds from nonbinding compounds by virtue of the opposite sign of their water-ligand nuclear Overhauser effects (NOEs). Preferably, the concentration of each compound in each sample is no greater than about 100 .mu.M, although higher concentrations can be used if desired.

Summary of Invention Paragraph (14):
[0013] In this method when binding is detected using the WaterLOGSY technique, extremely low levels of target can be used with ratios of ligand to target of about 100:1 to about 10:1. Preferably, the concentration of target molecule is no greater than about 10 .mu.M. More preferably, the concentration of target molecule is about 1 .mu.M to about 10 .mu.M. For data analysis, binding compounds are distinguished from nonbinders (i.e., nonbinding compounds) by the opposite sign of their water-ligand NOEs. With this method, there is no need to collect a reference spectrum in the absence of a target molecule.

Brief Description of Drawings Paragraph (21):
[0035] FIG. 16. Region of the 600.13 MHz WaterLOGSY spectrum of a compound mixture with added target protein. The concentration of protein was 10 .mu.M while the concentration of each compound was 100 .mu.M. The spectrum was acquired on a Bruker 5 mm flow-injection probe at 27.degree. C. A total of 4K scans were collected resulting in a total acquisition time of about 288 minutes. A mixing time of 2.0 seconds was used.

Brief Description of Drawings Paragraph (22):
[0036] FIG. 17. Comparison of WaterLOGSY spectrum (bottom panel) of thrombin with a compound mixture of the genomics screening library and the reference spectrum of DPS (top panel).

Detail Description Paragraph (5):
[0040] Thus, the present invention provides methods of identifying a compound that

binds to a target molecule (preferably, a protein) that are based on NMR spectroscopy techniques. Such methods typically involve the use of relaxation-editing techniques, for example, which involve monitoring changes in resonance intensities (preferably, significant reductions in intensities) of the test compound upon the addition of a target molecule. Preferably, the relaxation-editing techniques are one-dimensional, and more preferably, one-dimensional .sup.1H NMR techniques. Alternatively, such methods can involve the use of WaterLOGSY. This involves the transfer of magnetization from bulk water to detect the binding interaction. Using WaterLOGSY techniques, binding compounds are distinguished from nonbinders by the opposite sign of their water-ligand nuclear Overhauser effects (NOEs).

Detail Description Paragraph (6):
[0041] Important elements that contribute to the success of the methods of the invention preferably include developing a suitable small library of compounds to screen, carrying out the binding assay at low concentrations of target and near equimolar ratios of ligand to target (for relaxation-editing), or at extremely low concentrations of target (if desired) and higher ratios of ligand to target (for WaterLOGSY), and the capacity for rapid throughput of data collection. For example, for relaxation-editing NMR techniques, the concentration of target molecule is preferably no greater than about 1.0.times.10.sup.-4 M, and for WaterLOGSY NMR techniques, the concentration of target molecule is preferably no greater than about 10 .mu.M.

Detail Description Paragraph (9):
[0044] Carrying out a relaxation-editing binding assay (preferably, a 1D .sup.1H NMR assay) at low concentrations of target (preferably, no greater than about 1.0.times.10.sup.-4 M, and more preferably, no greater than about 5.0.times.10.sup.-5 M) and near equimolar ratios of ligand to target creates the requirement that compounds testing positive for binding have affinities within a factor of about 3-4 of this same concentration (preferably, having a dissociation constant of no less than about 2.0.times.10.sup.-4 M). A similar affinity threshold can be obtained by carrying out a WaterLOGSY based binding assay at even lower target concentrations (preferably, no greater than about 10 .mu.M, but is more preferably about 1 .mu.M to about 10 .mu.M) and ligand to target ratios of about 100:1 to about 10:1. This level of affinity is desired if the subsequent steps of focused screening and directed chemical elaboration are to be successful in elucidating a lead chemical template with very low affinity (e.g., one having a dissociation constant of at least about 1.0.times.10.sup.-6 M). Carrying out the initial screening at these low concentrations also avoids detection of unwanted compounds with much smaller dissociation constants in the 1.0.times.10.sup.-3 M range, which are less specific in their binding and therefore harder to turn into lead chemical templates given their weak affinity initially.

Detail Description Paragraph (16):
[0051] Sample requirements can be reduced even further if WaterLOGSY methods are used as an alternative to the relaxation-editing method described above to detect the binding interaction. WaterLOGSY is described in more detail in C. Dalvit et al., J. Biomol. NMR, 18, 65-68 (2000).

Detail Description Paragraph (17):
[0052] Since the WaterLOGSY experiment relies on the transfer of magnetization from bulk water to detect the binding interaction, it is a very sensitive technique. As such, the concentration of target molecule (e.g., protein) in each sample preferably can be reduced to no greater than about 10 .mu.M (preferably, about 1 .mu.M to about 10 .mu.M) while the concentration of each compound can be about 100 .mu.M. This results in ratios of target molecule to compounds in each sample reservoir of about 100:1 to about 10:1. The exact concentrations and ratios used can vary depending on the size of the target molecule, the amount of target molecule available, the desired binding affinity detection limit, and the desired speed of data collection. In contrast to the relaxation-editing method, there is no need to collect a comparison or control spectrum to identify binding compounds from nonbinders. Instead, binding compounds are distinguished from nonbinders by the opposite sign of their water-ligand nuclear Overhauser effects (NOEs).

Detail Description Paragraph (83):
[0109] Relaxation-Edited or WaterLOGSY-Based Flow-Injection NMR Screening Methods

Detail Description Paragraph (87):
[0113] When binding is detected using the WaterLOGSY technique, sample preparation and use of the flow-injection apparatus is identical, except that extremely low levels of target are used (1-10 .mu.M) with ratios of ligand to target of 100:1 to 10:1. For data analysis, binding compounds are distinguished from nonbinders by the opposite sign of their water-ligand NOEs. In contrast to the relaxation-edited technique, only a single WaterLOGSY spectrum is used for each ligand mixture. There is no need to collect a reference spectrum in the absence of target protein. An example is illustrated in FIG. 16 for a mixture of compounds and a different protein. In the WaterLOGSY spectrum shown in FIG. 16, binding compounds have resonances of opposite intensity (sharp positive peaks) than nonbinders (near zero intensity or sharp negative peaks). Residual protein resonances are also of positive intensity.

Detail Description Paragraph (90):
[0116] In our 1D relaxation-edited .sup.1H NMR data sets, one can simply identify the ligand resonances by inspection since their intensity is reduced in the presence of protein as shown in FIG. 14. In our WaterLOGSY data sets, binding compounds are distinguished from nonbinders by the opposite sign of their water-ligand NOEs as observed in FIG. 15. In either case, comparison to an assigned small molecule control spectrum are made to identify the compound associated with the indicated resonances.

Detail Description Paragraph (108):
[0134] NMR experiments for validating the functional genomics library with proteins of known functions were conducted on a Bruker Avance 600 MHz spectrometer equipped with 5 mm FISEI flow probe and Gilson 215 liquid sample handler. Binding was detected using the WaterLOGSY experiment.

Detail Description Paragraph (110):
[0136] The functional genomics screening library was screened against thrombin obtained from Sigma, which is one of the test proteins used for validation of the library. One assay mixture contained 133 .mu.M of N-alpha-dansyl-DL-tryptophan cyclohexylammonium salt (DPS) and 7 .mu.M of thrombin in 100 mM phosphate buffer, pH6.5. This mixture also contained Benzyl (S)-(-)-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (ZPR), Chymostatin A (CSA), Tetrahydrofolic acid (C2F), Haloperidol (THK). Referring to FIG. 17, the reference NMR spectrum of DPS is in the top panel while the WaterLOGSY spectrum of the mixture is shown in the bottom panel. The positive peak in the WaterLOGSY spectrum indicates binding of DPS to thrombin. The peaks indicated by red asterisks in the WaterLOGSY spectrum correspond to peaks from the reference spectrum of DPS shown in the top panel.

CLAIMS:

31. A method of identifying a compound that binds to a target molecule, the method comprising: providing a plurality of mixtures of test compounds, each mixture being in a sample reservoir; introducing a target molecule into each of the sample reservoirs to provide a plurality of test samples; providing a nuclear magnetic resonance spectrometer equipped with a flow-injection probe; transferring each test sample from the sample reservoir into the flow-injection probe; collecting a WaterLOGSY nuclear magnetic resonance spectrum on each sample in each reservoir; and analyzing the spectra of each sample to distinguish binding compounds from nonbinding compounds by virtue of the opposite sign of their water-ligand NOEs.

40. The method of claim 31 wherein collecting a WaterLOGSY nuclear magnetic resonance spectrum comprises collecting a 1D WaterLOGSY nuclear magnetic resonance spectrum.

46. A method of identifying a protein function, the method comprising: providing a plurality of mixtures of test compounds consisting of known inhibitors, cofactors, and substrates of known proteins, each mixture being in a sample reservoir and

containing a plurality of test compounds; introducing a target molecule into each of the sample reservoirs to provide a plurality of test samples; providing a nuclear magnetic resonance spectrometer equipped with a flow-injection probe; transferring each test sample from the sample reservoir into the flow-injection probe; collecting a WaterLOGSY nuclear magnetic resonance spectrum on each sample in each reservoir; comparing the spectra of each sample to the spectra taken under the same conditions in the absence of the target molecule to identify compounds that bind to the target molecule, wherein the concentration of target molecule and each compound in each sample is no greater than about 5 .mu.M and 125 .mu.M, respectively; and determining a function of the target molecule based upon the test compounds that bind to the target molecule.